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ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF SIDDHA DRUG ATTATHI CHOORANAM (ATC)

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ABSTRACT

Attathi chooranam (ATC) is a classical Siddha herbo-mineral drug. The aim of this research was to find out the antibacterial activity of ATC against the five gram-positive, five gram-negative bacteria and anti-fungal activity of five fungi in different extracts. The ethanol extracts of 50 µℓ and 100 µℓ of ATC showed the highest antimicrobial activity against all the positive and negative tested bacteria and fungi when compared to aqueous extracts. 100 µℓ of ethanol extract of ATC has high activity against gram-positive and gram-negative bacteria and fungi than 50 µℓ of ethanol extract. The highest anti-bacterial activity recorded 100 µℓ and 50 µℓ of ethanol in gram-negative bacteria *Klebsilla pneumonia* (25 mm & 22 mm), *Pseudomonas aeruginosa* (26 mm &18 mm) and gram-positive bacteria *Staphylococcus aureus* (24 mm & 16 mm), *Streptococcus mutans* (22 mm & 20 mm) which were compared with positive control of Streptomycin. All extracts of ATC have high activity against gram negative bacteria compared with gram positive bacteria. ATC extracts of 100 µℓ and 50 µℓ of ethanol exhibited potential antifungal activity against *Aspergillus niger* (18 mm & 14 mm) compared with positive control (fluconazole). According the 100 µℓ and 50 µℓ of aqueous extracts of ATC kept antifungal activity against *Penicillium notatum* (18mm & 15 mm) and *Candida Albicans* (15 mm & 11 mm) meanwhile activity has not been reported against *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifera*.

KEYWORDS: Anti-bacterial activity, Anti-fungal activity, Attathi chooranam, Disc diffusion method.

1. INTRODUCTION

It has been described that the herbs and drugs were used for the elimination of microorganisms in the ancient philosophies. Many plants derivatives such as spices, fruit preparations, vegetable preparations or extracts have been used for centuries for the preservation and extension of the shelf life of foods (Chattopadhyay and Bhattacharyya, 2007). Increasing the resistance of microorganisms encouraged to find out new drugs. Antimicrobial activity is the process of killing or inhibiting the diseases causing microbes such as Bacteria, Fungai and Viruses. Various antimicrobial agents are used for the purpose in medicines and but microbes are producing resistance against antimicrobial agents. Therefore, there is need to discover new infection-fighting agents to control microbial infections (Sieradzki et al, 1999).

Attathi chooranam (ATC) is a classical Siddha herbo-mineral drug and which could be used in *Vatha* related *Soothaka noikal* (Ovarian disorders). (*Anupoka vaithiya navaneetham* (part-8)). Atta means eight and athi means first, original, root, extra and etc. ATC has seven medicinal plant parts such as *Piper longum* Linn., *Zingiber officinale* Roscoe., *Nigella sativa* Linn., *Cuminum cyminum* Linn., *Piper nigrum* Linn., *Ferula asafoetida* Linn., and *Carum coptium* Benth & Hook.F. and one mineral substance as Sodium chloride impure with equal quantity of *Saccharum officinarum*.

The aim of this study is to evaluate the antimicrobial activity of Siddha medicine ATC used for various extracts. ATC of ethanol and aqueous extracts in different concentrations 50 µℓ and 100 µℓ were used for the study of anti-bacterial activity of five gram positive bacteria such as *Staphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius, Bacillus subtilis, Streptococcus mutans* and five gram negative bacteria such as *Klebsilla pneumonia, E.coli, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis* and anti-fungal activity against *Aspergillus flavus, Aspergillus niger, Penicillium notatum, Rhizopus stolonifer* and *Candida Albicans*.

2. MATERIALS AND METHODS

2.1. Collection and Identification of plant materials

The ingredients of drug were collected from Siddha medical shop Tirunelveli, Tamil Nadu, India and authenticated by the Professors of department of Gunapadam and Medicinal Botany at Government Siddha Medical College and Hospital, Palayamkottai.

2.2. Purification of raw drugs

The raw drugs were purified as per the methods mentioned in the Siddha literature (Sarakku Suthi Muraigal).

Table 1: Ingredients of drug ATC.

No	Ingredients	Botanical name	Family name	Part used	Quantity
1	*Thippili*	*Piper longum* Linn.	Piperaceae	Dry fruit	8 parts
2	*Sukku*	*Zingiber officinale* Roscoe.	Zingiberaceae	Dry rhizhome	7 parts
3	*Karujcheeragam*	*Nigella sativa* Linn.	Ranunclaceae	Seeds	6 parts
4	*Natseeragam*	*Cuminum cyminum* Linn.	Apiaceae	Seeds	5 parts
5	*Milaku*	*Piper nigrum* Linn.	Piperaceae	Dry fruit seeds	4 parts
6	*Inthuppu*	Sodium chloride impure (Chemical name)			3 parts
7	*Perumkayam*	*Ferula asafetida* Linn.	Umbelliferae	Gum resin	2 parts
8	*Omam*	*Carum coptium* Benth & Hook.F.	Apiaceae	Seeds	1 part
9	*Sarkarai*	*Saccharum officinarum*	Poaceae	Jaggery	36 parts

2.3. Preparation of the drug *Attathi chooranam* (ATC)

All the crude drugs were dried well in shadow and made into micronized powder separately. Then these were mixed with mentioned proportions in table No.1.

2.4. Antimicrobial activity procedure

The research was carried out in the department of Inbiotics, Institute of biology and clinical research, William hospital campus, Nagercoil, Tamil Nadu, India during December 2019

2.4.1. Antibacterial Activity Procedure

Dilution: 1 mg in 1 mℓ

Test Organism

The test microorganisms used for antimicrobial analysis Microbial strains were purchased from Microbial Type Culture Collection and Gene Bank (**MTCC**) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37 ºC for 24 hours. Inoculum was prepared by aseptically adding the fresh culture into 2 m ℓ of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/mℓ. Standardized inoculum used for Antimicrobial test.

Antimicrobial Test

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 mℓ of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121 ^{O}C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured Petri plates (25 mℓ /plate) the plates were swabbed with Pathogenic Bacteria culture. Finally, the Sample or Sample loaded Disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37 °C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in milli meters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010).

2.4.2. Anti-fungi assay by disc diffusion method (Bauer *et al*., 1966)

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Fungi strains were swabbed using sterile cotton swabs in SDA agar plate. Up to 40 µℓ of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22 °C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres.

3. RESULTS

Siddha drug ATC were evaluated for their antimicrobial potential against five gram-positive bacteria strains, five gram-negative bacteria strains and five fungi strains microorganisms in this study. Tables 2,3 & 4 summarizes the results obtained.

3.1. Antibacterial activity of gram-positive bacteria

A. Ethanol extract of ATC

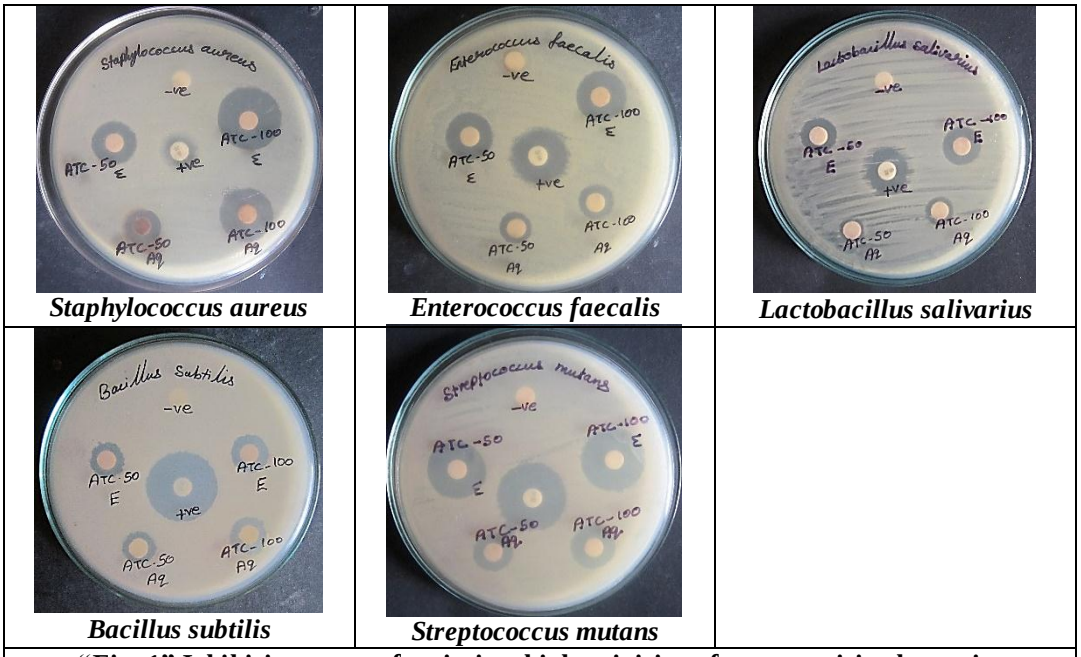
The ethanol extract of 50 µℓ showed maximum zone of inhibition 20 mm against *Streptococcus mutans*, followed by *Enterococcus faecalis* 18 mm, *Staphylococcus aureus* 16 mm, 13 mm against *Lactobacillus salivarius* and *Bacillus subtilis* as shown in table 2.

The ethanol extract of 100 µℓ showed extreme zone of inhibition in G+ bacteria *Staphylococcus aureus* 24 mm, *Streptococcus mutans* 22 mm, *Enterococcus faecalis* 21 mm followed by 15 mm against *Lactobacillus salivarius* and *Bacillus subtilis* as shown in table 2.

Table 2: Antibacterial activity of ethanol and aqueous extracts of gram-positive bacteria.

Sample Code	Gram Positive Bacteria Strains Name				
	***Staphylococcus aureus* (G+) MTCC 916 (mm)**	***Enterococcus faecalis* (G+) MTCC 439 (mm)**	***Lactobacillus salivarius* (G+) MTCC 1026 (mm)**	***Bacillus subtilis* (G+) MTCC 1134 (mm)**	***Streptococcus mutans* (G+) MTCC 916 (mm)**
ATC.E. 50	16	18	13	13	20
ATC. E. 100	24	21	15	15	22
ATC. Aq. 50	14	12	8	11	14
ATC. Aq.100	19	14	10	14	16
PC	14	23	18	26	21
NC	-	-	-	-	-

PC (Bacteria): Positive control (Streptomycin), NC: Negative (plain disc), (-): No Zone, mm: millimeter, G+ : Gram Positive, E: Ethanol extract, Aq: Aqueous extract.



Staphylococcus aureus ***Enterococcus faecalis*** ***Lactobacillus salivarius***

Bacillus subtilis ***Streptococcus mutans***

"Fig. 1" Inhibition zones of antimicrobial activities of gram-positive bacteria.

B. Aqueous extract of ATC

The aqueous extract of 50 µℓ showed maximum zone of inhibition 14 mm against *Streptococcus mutans* and *Staphylococcus aureus* followed by *Enterococcus faecalis* 12 mm and *Bacillus subtilis* 11 mm. Minimum zone of inhibition against 8 mm *Lactobacillus salivarius* as shown in table 2.

The aqueous extract of 100 µℓ displayed maximum zone of inhibition *Staphylococcus aureus* 19mm, *Streptococcus mutans* 16mm, followed by 14 mm against *Enterococcus faecalis* and *Bacillus subtilis*. *Lactobacillus salivarius* presented 10 mm that was minimum inhibition zone as shown in table 2.

Table 3: Antibacterial activity of ethanol and aqueous extracts of gram-negative bacteria.

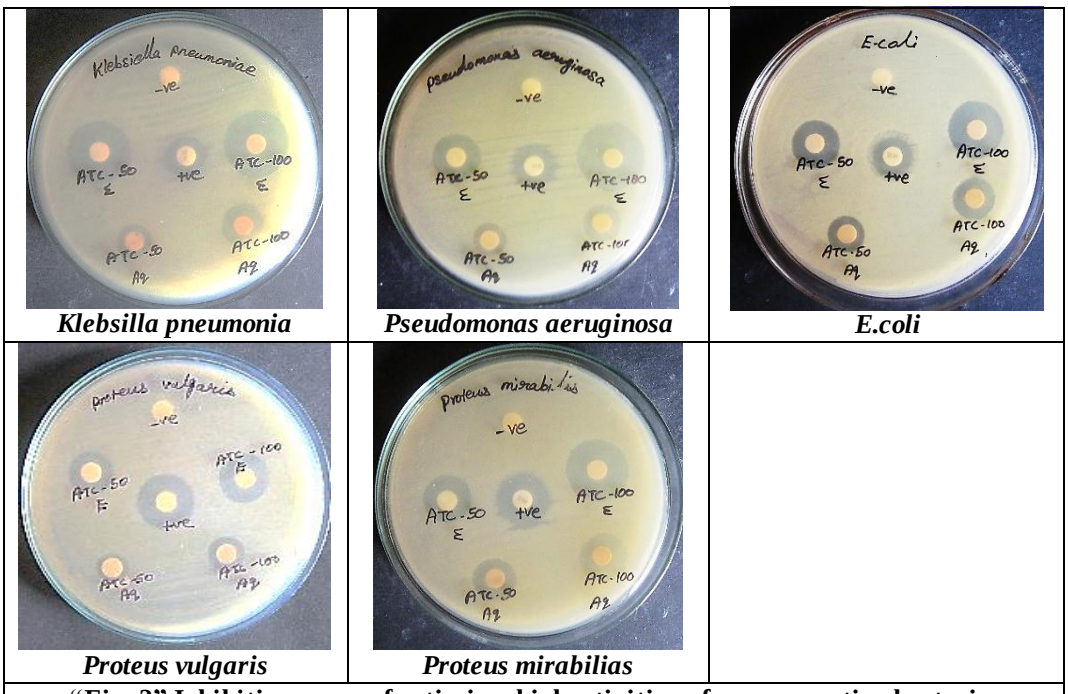
Sample Code	Gram Negative Bacteria Strains Name				
	***Klebsilla pneumonia* (G-) MTCC 530 (mm)**	***E.coli* (G-) MTCC 1671 (mm)**	***Pseudomonas aeruginosa* (G-) MTCC 741 (mm)**	***Proteus vulgaris* (G-) MTCC 426 (mm)**	***Proteus mirabilis* (G-) MTCC 1429 (mm)**
ATC.E. 50	22	17	18	14	17
ATC.E. 100	25	19	26	17	21
ATC. Aq. 50	13	13	13	11	14
ATC. Aq.100	16	16	15	13	18
PC	14	17	17	18	19
NC	-	-	-	-	

PC (Bacteria): Positive control (Streptomycin), NC: Negative (plain disc), (-): No Zone, mm: millimeter, G-: Gram Negative.

3.2. Antibacterial activity of gram-negative bacteria

A. Ethanol extract of ATC

The tested drug ATC of ethanol extract of 50 µℓ showed antibacterial activity against Gram negative bacteria by the zone of inhibition of *Klebsilla pneumonia* 22 mm, *Pseudomonas aeruginosa* 18 mm, 17 mm *E.coli* and *Proteus mirabilias* in this study. The least activity observed against *Proteus vulgaris*14 mm. Meanwhile the tested drug ATC of ethanol extract of 100 µℓ exhibited potential antibacterial activity of Gram negative bacteria by the zone of inhibition of *Pseudomonas aeruginosa* 26 mm, followed by *Klebsilla pneumonia* 25 mm, *Proteus mirabilias* 21 mm, *E.coli* 19 mm and *Proteus vulgaris* 17 mm of poor activity (Table 3).



Klebsilla pneumonia ***Pseudomonas aeruginosa*** ***E.coli***

Proteus vulgaris ***Proteus mirabilias***

"Fig. 2" Inhibition zones of antimicrobial activities of gram-negative bacteria.

B. Aqueous extract of ATC

The aqueous extract of 50 µℓ showed maximum zone of inhibition 14 mm against *Proteus mirabilis* and equal zone of inhibition 13 mm against inhibition *Klebsilla pneumonia*, *E.coli and Pseudomonas aeruginosa*. Minimum zone of inhibition *Proteus vulgaris* 11 mm as shown in table 3.

The aqueous extract of 100 µℓ displayed maximum zone of inhibition *Proteus mirabilis* 18 mm, followed by 16 mm against *Klebsilla pneumonia* and *E.coli*. *Pseudomonas aeruginosa* presented 15 mm and minimum inhibition zone of inhibition 13 mm in *Proteus vulgaris* as shown in table 3.

Table 4: Antifungal activity of ethanol and aqueous extracts of fungi strains.

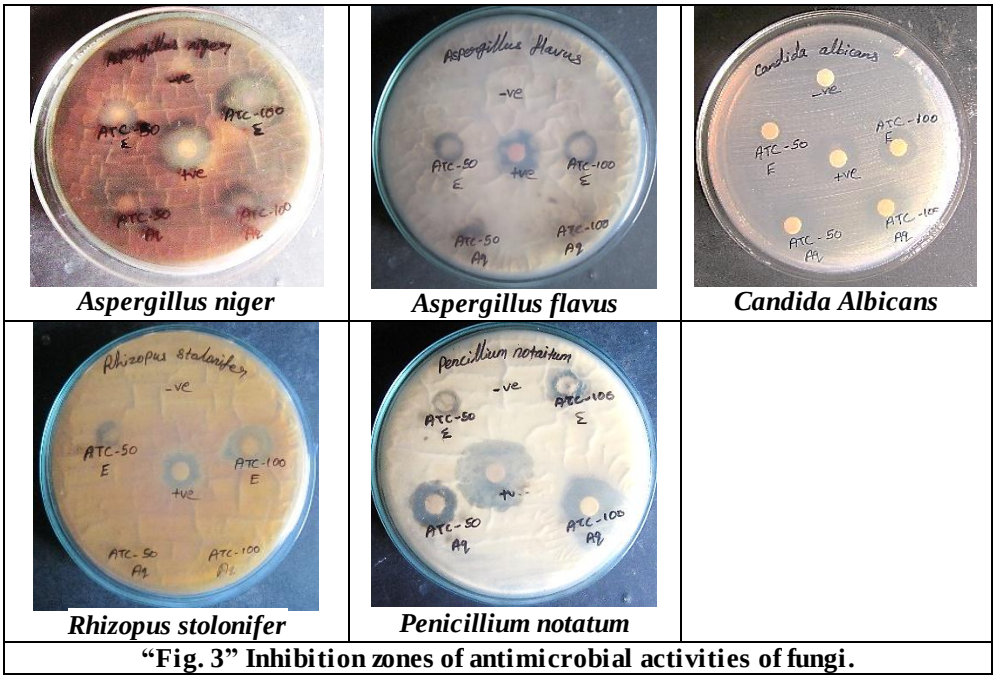
Sample Code	*Fungi Strains Name*				
	***Aspergillus flavus* (F) MTCC 535 (mm)**	***Aspergillus niger* (F) MTCC 281 (mm)**	***Penicillium notatum* (F) MTCC 2647 (mm)**	***Rhizopus Stolonifer (F)* MTCC 162 (mm)**	***Candida Albicans* (F) MTCC 183 (mm)**
ATC.E. 50	9	14	9	7	8
ATC.E. 100	11	18	13	13	13
ATC. Aq. 50	-	-	15	-	11
ATC. Aq.100	-	-	18	-	15
PC	15	16	22	15	16
NC	-	-	-	-	-

PC (Fungi): Positive control (fluconazole), NC: Negative (plain disc), (-): No Zone, mm: millimeter.

3.3. Antifungal activity

A. Ethanol extract of ATC

According to this study that the tested drug ATC extracts of ethanol 50 µℓ showed antifungal activity against *Aspergillus niger* 14 mm, *Aspergillus flavus* and *Penicillium notatum* 9 mm, *Candida Albicans* 8 mm and *Rhizopus stolonifer* 7 mm. At the same time ATC extracts of ethanol 100 µℓ showed potential antifungal activity against *Aspergillus niger* 18 mm followed by 13 mm againts *Penicillium notatum*, *Rhizopus stolonifer* and *Candida Albicans*. *Aspergillus flavus* 11mm that showed poor activity as shown in table 4 and figure 3.



"Fig. 3" Inhibition zones of antimicrobial activities of fungi.

B. Aqueous extract of ATC

Although aqueous extracts of ATC 50 µℓ and 100 µℓ showed the antifungal activity against *Penicillium notatum* 15 mm & 18 mm and *Candida Albicans* 11 mm & 15 mm, but *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus stolonifer did not show.*

4. DISCUSSIONS

In the alternative methods of using plant material to control pathogenic microorganism has been interested recently (Aqil et. al., 2005) and plants products have been shown resistant against pathogenic bacteria (Nostro et. al., 2006). The emergence of multidrug resistant strain of many pathogens is a serious threat and makes more difficult to cure diseases. The development of effective natural and non-toxic drug for treatment must be directed towards. (Chandra 2013). This study also explained the antimicrobial property of Siddha drug ATC.

In this research the antibacterial activity of ATC were checked against five gram positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Lactobacillus salivarius*, *Bacillus subtilis* and *Streptococcus mutans* and five gram negative bacteria *Klebsilla pneumonia*, *E.coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis*. The antifungal activities were tested against *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer* and *Candida Albicans*.

The ethanol extracts of the ATC showed significant activity against all the tested positive and negative

bacteria and fungi compared to aqueous extract. The ethanol extract of 100 µℓ of ATC has high activity of gram positive and gram negative bacterial and fungal against ethanol extract of 50 µℓ. Similarly, the aqueous extract of 100 µℓ of ATC has high gram positive and gram negative bacterial and fungal activity against aqueous extract of 50 µℓ.

Ethanol extract 100 µℓ of ATC showed high zone of inhibition against gram positive bacteria *Staphylococcus aureus* (24mm) and *Streptococcus mutans* (22mm) and lower against *Bacillus subtilis* (15mm) compare with positive control. The highest antibacterial activity recorded in *Staphylococcus aureus* (19 mm) in aqueous extract of 100 µℓ and equal activity (14 mm) in aqueous extract of 50 µℓ which was compared with positive control (14 mm) (Streptomycin) (Table 2).

The ethanolic extract of 100 µℓ ATC gave good result against the *Klebsilla pneumonia* (25mm) and *Pseudomonas aeruginosa* (26mm) compared with positive control 14 mm & 17 mm and there were no less effective except *Proteus vulgaris* (17mm) against positive control (Streptomycin). *Klebsilla pneumonia* (16 mm) highest activity in aqueous 100 µℓ compared with positive control (14 mm) (Streptomycin) (Table 3).

The highest antifungal activity recorded in ethanol extract 100 µℓ of *Aspergillus niger* (18mm) compared with positive control (fluconazole). Aqueous extracts of ATC 50 µℓ and 100 µℓ possessed antifungal activity against *Penicillium notatum* and *Candida Albicans* and activity has not been reported against *Aspergillus flavus, Aspergillus niger* and *Rhizopus stolonifer* (Table 4).

5. CONCLUSIONS

The overall results of this study showed that the 50 µℓ and 100 µℓ ethanolic and aqueous extracts of ATC has high zone of inhibition against gram negative bacteria (*Klebsilla pneumonia, E.coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Proteus mirabilis*.) compare with gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius, Bacillus subtilis* and *Streptococcus mutans*) with positive control (Streptomycin).

The tested drug ATC extracts of ethanol possessed antifungal activity against *Aspergillus niger Aspergillus flavus, Penicillium notatum, Rhizopus stolonifer* and *Candida Albicans*. Aqueous extracts of ATC 50 µℓ and 100 µℓ possessed antifungal activity against *Penicillium notatum* and *Candida Albicans* and activity has not been reported against *Aspergillus flavus, Aspergillus niger* and *Rhizopus stolonifera*.

It is concluded that this study would exhibit some valuable compound that has to be used to more potential antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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